Docket No. 128.1040

UNITED STATES PATENT AND TRADEMARK OFFICE

	Examiner:	HUBBERT, Catherine, S. Art Unit: 1636
Re:	Application of:	NALDINI, Luigi et al.
	Serial No.:	10/554,181
	Filed:	December 27, 2005
	For:	**LENTIVIRAL VECTORS CARRYING SYNTHETIC BI-DIRECTIONAL PROMOTERS AND USES THEREOF**
	Confirmation No.	1875

DECLARATION OF LUIGI NALDINI UNDER 37 CFR § 1.132

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

October 8, 2010

Sir:

I, Luigi Naldini, hereby declare and state as follows:

1. I am a co-inventor of the above-identified instant patent application.

2. I graduated from the University of Turin, Italy, where I received a Masters degree in Medicine in July, 1983. I am presently Director of the San Raffaele Telethon Institute for Gene Therapy, San Raffaele Foundation, Milan, Italy. My further qualifications and publications are set forth by my *Curriculum Vitae*, that is attached to this Declaration as Exhibit 1.

3. I am familiar with the specification and claims of the present patent application, and I have read the office action. I am aware that the Examiner has taken the position that claims 1, 3-4 and 6-18 lack novelty and that claim 5 would have been obvious, over the references cited by the Examiner in the office action. In the interest of advancing the examination of the present patent application, I provide the following expert analysis of each of these references, relative to the pending claims.

4. The Examiner alleges that claims 1, 3-4 and 6-15 lack novelty over Chtarto et al. ("Chtarto"; US6,780,639) under 35 USC 102(e). Chtarto teaches a bidirectional promoter, but nowhere teaches or suggests that their bidirectional promoter include a "promoter sequence of an animal gene comprising an enhancer region," as required by claim 1. For example, see Chtarto at Fig. 6 and Cols 4-6. The bidirectional promoter described by Charto is based on a prokaryotic tetracycline (Tet) responsive system. Once exogenously provided with a tetracycline-regulated transcriptional factor and the administration of doxycycline, the described promoter can express the genes of interest in mammalian cells. This approach reconstitutes a prokaryotic transcriptional switch in animal cells and requires the co-expression of prokaryotic transcription control elements together with prokaryotic transcription factors. Given the substantial differences accrued during evolution in the genome expression mechanisms and controls between prokaryotic and eukaryotic organisms, one cannot extrapolate from the work by Charto whether a synthetic bidirectional design made of eukaryotic transcriptional control elements and operated by endogenous eukaryotic transcription factors would support bidirectional transcription. It is my expert opinion that the Chtarto bidirectional promoter, that lacks a promoter sequence of an animal gene comprising an enhancer region, would therefore be unable to meet the additional

requirements of claim 1, for "coding sequences in opposite direction in animal cells..." and that the subject promoter be "endogenously regulated."

5. The Examiner alleges that claims 1, 3-4 and 6-11 and 13-18 lack novelty over Itoh et al. ("Itoh"; US6,995,011) under 35 USC 102(e). Itoh teaches a bidirectional promoter but nowhere teaches or suggests a "promoter sequence of an animal gene animal gene comprising an enhancer region," as required by claim 1. Itho clearly states that the object of their invention is : "to provide an expression control system having all of the following features:... 2) a system in which the gene of interest can be removed at any time after changing the nature of cells, by administering a low molecular weight compound to the cell;". This is in complete contrast with our claimed invention providing endogenously regulated bidirectional promoters, that are not dependent on administration of exogenous drugs to regulate expression.

6. In particular, the bidirectional promoters described in Itoh et al. are bidirectional Tet-responsive promoter (Figure 2) and low molecular weight compound-responsive bidirectional promoter (Figure 3), examples of the low molecular weight compound-responsive bidirectional promoter are shown in Figure 5. The low molecular weight compound-responsive bidirectional promoter of Itoh et al. has a positive feedback system in which a low molecular weight compound responsive transactivator is placed on one side of a low molecular weight compound-responsive bidirectional promoter under the control thereof (specifically, a DNA encoding rtTA is placed on one side of a bidirectional Tet-responsive promoter under the control thereof). It is my expert opinion that the Itoh bidirectional promoter would therefore be unable to meet the additional requirement of claim 1, for "coding sequences in opposite direction in animal cells..."

The Itoh bidirectional promoter also shows no evidence of teaching or suggesting coordinate transcription, as required by claim 1.

7. The Examiner alleges that claims 1-4 and 7-8, 10 and 14 lack novelty over Fux et al. ("Fux") under 35 USC 102(b). Fux teaches several plasmids carrying two completely independent promoters. Each promoter has different activity and different inducibility. Nowhere does Fux teach or suggest a bidirectional promoter as required by claim 1, that provides for coordinate transcription of coding sequences in the opposite orientation. This is confirmed, for example, by Fux, at page 110, left column, first paragraph, and page 114, left column, last paragraph, that describes a "dual-regulated expression technology" with vectors designated as pDuoRex 7 and pDuoRex 8, respectively. For example, the "stuffer fragment" mentioned at page 114, last paragraph, is employed to, "limit hang-over co-activation" and make sure that the "repressed expression unit" is not expressed or co-activated when the other expression unit is activated. Thus, coordinate transcription, in the opposite direction, which is required by claim 1, is simply not possible, by design, with the Fux dual-regulated expression technology.

8. In particular, according to Fux, "pDuoRex1-based expression configurations exemplify that efficient dual-regulated expression of two independent gene activities is indeed feasible" (e.g., Fux, p114, left column), while our claimed vector is designed in order to maximize co-ordinate expression. Indeed, these two results are distinct and even opposite in outcome. In addition, dual regulated expression in the Fux pDuoRex vectors requires exogenous transactivator molecules (tTA and PIT) that are antibiotic regulated. In this context, the noncoding stuffer fragments or chicken HS4 (cHS4) insulators (Figure 1, legend) have been

cloned between divergently oriented expression units to minimize interference. The artificial promoters (PPIR or PPIR8; PhCMV*-1), are assembled by cloning PIT- or tTA-specific operators (pir; tetO), adjacent to a minimal eukaryotic promoter (Phsp70min for PPIR; PhCMVmin for PPIR8 and PhCMV*-1), in an antibiotic-adjustable manner (page 109)

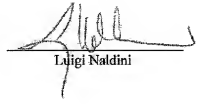
9. It should also be appreciated that PIT- or tTA-specific operators (pir; tetO) are synthetic sequences derived from prokariotic sources, and cannot be regarded as endogenously regulated enhancer regions[1] since they are completely inert in the absence of the synthetic transactivator partner (PIT or tTA). Unlike the instantly claimed bidirectional promoter, all of the Fux DuoRex-based, dual-regulated bidirectional promoters require concomitant production of exogenous transactivators molecule, tTA and PIT (page 114 first paragraph). In conclusion, in my expert opinion, the pDuoRex expression constructs, such as those described in table 1 and figure 1 of the Fux reference, are clearly different from the bidirectional promoters of the instantly claimed invention.

10. The Examiner alleges that claim 5 would have been obvious over Chtarto or Itoh, either in view of Hope et al. ("Hope"; US6,136,597) under 35 USC 103(a). Claim 5 is cancelled, and the limitations of claim 5 are now included in claim 4. Claim 4 depends from claim 1, and therefore includes all of the elements of claim 1. The Examiner concedes that neither Chtarto or Itoh teach inclusion of a post-transcriptional regulatory element positioned upstream to one or each of the poly A sites. In addition, as explained above, Chtarto fails to teach a bidirectional

[1] The artisan is well aware of the meaning of "enhancer." An enhancer is a short region of DNA that can be bound with proteins (namely, the trans-acting factors, much like a set of transcription factors) to enhance transcription levels of genes (hence the name) in a gene cluster. See, e.g., Glossary of Genetics, Classical and Molecular, by R. Rieger et al., Fifth Ed., Springer-Verlag, published 1991, page 167, attached as Exhibit 2 for the convenience of the Examiner.

promoter comprising a "promoter sequence of an animal gene animal gene comprising an enhancer region," as required by claim 1, and therefore by claim 4. Further, Itoh fails to teach a divalent promoter according to claim 1, that includes an animal promoter element, and would therefore be unable to meet the additional requirement of claim 1, for "coding sequences in opposite direction in animal cells..." Even if Hope teaches that inclusion of a post-transcriptional element, such as a WPRE[2], can enhance expression of a transgene in a target cell, it is clear that Hope fails to remedy any of the remaining deficiencies in the primary references. Thus, even if the alleged combination of references is made, the result could not be the bidirectional promoter required by claim 4.

11. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 USC 1001 and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Dated: This 8 day of October, 2010. By: 

Luigi Naldini

[2] Woodchuck Hepatitis B Virus Posttranscriptional Regulatory Element

Exhibit 1

Curriculum Vitae

NAME	POSITION TITLE
Luigi NALDINI	*Professor* of Cell and Tissue Biology and *Professor* of Gene and Cell Therapy, "Vita Salute San Raffaele" University School of Medicine, Milan *Scientific Director*, San Raffaele Telethon Institute for Gene Therapy, Milan, Italy *Member* of Strategic Committee for Research, San Raffaele Institute, Milan, Italy

EDUCATION/TRAINING

INSTITUTION	DEGREE	YEAR(s)	FIELD OF STUDY
University of Torino Medical School	M.D.	1983	
University of Rome " La Sapienza"	Ph.D.	1983 - 1987	Cell and Developmental Biology
Meloy Laboratories (with Yossi Schlessinger), Rockville, MD	Post-doctoral training	1987 - 1989	Tyrosine Kinases and Signal Transduction

EMPLOYMENT AND EXPERIENCE

1990-96 Assistant Professor of Cell and Tissue Biology, Dept. of Biomedical Sciences and Oncology, University of Torino Medical School (since 1993 with tenure).

1994-96 Visiting Scientist, The Laboratory of Genetics (Director: Inder M. Verma), the Salk Institute for Biological Studies, La Jolla, CA.

1996-98 Senior Scientist and Director, Lentiviral Vector Project, Somatix Therapy Corp. & Cell Genesys, Foster City, CA.

1998-02 Associate Professor of Cell and Tissue Biology, University of Torino Medical School and Head, Laboratory for Gene Transfer and Therapy, Institute for Cancer Research, Candiolo (Torino), Italy.

Since 2002 Full Professor of Cell and Tissue Biology, "Vita Salute San Raffaele" University School of Medicine, Scientific Codirector San Raffaele Telethon Institute for Gene Therapy, Milan, Italy.

Since 2005 Full Professor with tenure

Since 2008 Scientific Director, San Raffaele Telethon Institute for Gene Therapy, Milan, Italy.

PROFESSIONAL ACTIVITIES

- Member of the Board of Directors (2005-2008) and of the Advisory Council (since 2008) of the American Society of Gene and Cell Therapy (ASGCT)
- Member of the Board of the European Society of Gene and Cell Therapy (ESGCT) (since 2008)
- Scientific Advisor on EMEA and WHO Committees for the evaluation of novel gene transfer medicines
- Served on several Scientific Committees of the ASGCT, ESCGT, American Association of Cancer Research (AACR), International Society for Stem Cell Research (ISSCR), International Society of Cell Therapy (ISCT)
- Associate Editor: *Human Gene Therapy*, Mary Ann Liebert Publisher
- Member of the Editorial Board: *Molecular Therapy, Gene Therapy*

PATENTS

Inventor of 12 granted international patents and 5 pending. These include a cornerstone patent on lentiviral vector technology owned by the Salk Institute and a family originator patent owned by Cell Genesys. Intellectual property more recently generated at the San Raffaele Institute covers bidirectional vectors for coordinate gene expression, micro-RNA regulated vectors, angiogenic monocytes and tolerogenic vectors.

HONORS

Elected Member of *EMBO*, the *European Molecular Biology Organization*, in 2008

Awarded the European Research Council (ERC) *Advanced Investigator Grant*, which recognizes top EU scientists with an outstanding achievement track record, in 2009

Keynote Speaker at:

- Keystone Symposia on "The Potent New Anti-Tumor Immunotherapies", Banff, Canada (03/07)
- XIII Meeting of the International Society for Cell Therapy (ISCT), Sidney, Australia (06/07)
- XI Meeting of the ASGCT, Boston, USA (05/08)
- XVI Meeting of the ESGCT, Brugge, Belgium (11/08)
- In Vivo Barriers to Gene Delivery Meeting, Cold Spring Harbor Laboratory, USA (11/09)
- 4th European Congress of Virology, Cernobbio, Italy (04/10)

Research abstracts submitted from L. Naldini's laboratory to the ASGCT and/or ESGCT Annual Meetings were selected among the top abstracts at the 1999, 2000, 2001, 2003, 2004, 2005, 2006, 2007, 2008,2009 and 2010 annual conferences.

SCIENTIFIC ACTIVITIES

Has published 152 papers in international scientific journals (Total Impact Factor 1,479 based on Journals IF 2009, with average I.F.= 9.73 *per* paper). Overall, his papers have been cited >14,940 times since 1996 (as of August 2010). Scopus "*h*" index: 54.
Invited speaker or lecturer to more than 150 International Meetings, Workshops or Universities in the last 10 years. Keynote speaker in 13 venues in the last 2 years.

In his early career, L. Naldini identified the ligand for the Met receptor with Hepatocyte Growth Factor (HGF), proved its identity with Scatter Factor and elucidated its mechanism of regulation and function in triggering motility and invasion of epithelial cells. *MET* has since been one of the most investigated oncogene in epithelial cancer and metastasis.
During his stay within Inder Verma and Didier Trono laboratories at the Salk Institute for Biological Studies, La Jolla (1994-96), he first described the use of HIV-derived hybrid lentiviral vectors for gene transfer into non-dividing cells. The original paper reporting this work is one of top-cited articles in the journal Science (2,207 citations). He then developed the technology for safe and efficient use working as a senior scientist at Cell Genesys, Foster City, CA. He discussed with NIH, FDA and EMEA the requirements and implications of lentiviral vector administration to humans. Overall, this work laid the foundation for the currently broad use of lentiviral vectors; what was initially received as an elegant proof-of-principle of an unlikely and fearsome technology, has now become one of the most widely used tool in biomedical research.
At the end of 1998, L. Naldini returned to academia as professor at the University of Torino and in 2003 moved to the San Raffaele Telethon Institute for Gene Therapy in Milan, initially as co-director with Maria Grazia Roncarolo and since 2008 as director of the Institute.

Throughout this time he has continued to investigate new gene transfer approaches and exploit them to gain insights into fundamental biological processes of high relevance for molecular medicine, such as stem cell activity and angiogenesis, and to develop new therapeutic strategies for genetic disease and cancer.

Together with other laboratories, Naldini's work has shown the proficiency of lentiviral vectors at marking hematopoietic stem cells of mice and humans. By reaching exhaustive cell marking with minimal interference with cell function, individual stem cell activity can now be monitored *in vivo* to unprecedented levels. An unexpected boost towards the broad use of lentiviral vectors came from studies primarily conducted in Naldini's laboratory showing that the advanced design of lentiviral vectors is associated with much lower genotoxicity than conventional gamma-retroviral vectors, thus providing for a safer gene transfer platform despite the original concerns raised by the nature of the parental virus. The demonstration of high gene transfer efficiency coupled with improved safety provided by these studies has been crucial for moving lentiviral vectors to the clinic.

L. Naldini's efforts towards improving gene transfer have always been pursued with the clear goal in mind of therapeutic translation. He selected lysosomal storage disorders as paradigmatic diseases for testing the new therapeutic potential offered by lentiviral vectors. His work showed that the post-transplant recruitment of hematopoietic cells to the resident microglia pool can be exploited to deliver gene therapy to the central and peripheral nervous system, and treat metachromatic leukodystrophy (MLD) in the mouse model. A lentiviral vector based clinical trial for the human disease, which is invariably lethal and currently without any effective treatment, is now undergoing at the San Raffaele Institute. The successful first clinical testing of lentiviral vectors in hematopoietic stem cell gene therapy was reported in *Science* in Nov 2009 by a French team led by Patrick Aubourg and Nathalie Cartier, using the vector design previously developed by Naldini and collaborators.

The procedure was applied to the treatment of X-linked adrenoleukodystrophy, a disease closely related to MLD. *Science* welcomed the successful ALD study as a "Comeback for Gene Therapy" and included it among the top scientific breakthroughs of the year 2009.

By tracking the hematopoietic cell contribution to angiogenesis, Naldini's work established a novel paradigm in which the bone marrow contributes essential paracrine regulators to the newly formed vessels. These studies helped defining a new lineage of proangiogenic monocytes, which selectively engage in tissue remodeling and regeneration and can be distinguished from conventional monocytes by gene expression, surface markers and functional properties. Naldini and his collaborators are now exploiting these findings to develop a new therapeutic strategy by which the progeny of transplanted hematopoietic progenitors is engineered to selectively target gene therapy to tumors, thus enhancing therapeutic efficacy and avoiding systemic toxicity.

In another recent development, Naldini's research applied microRNA regulation to vector design and provided the prototype for making transgenes and medically used viruses stringently responsive to cell type- and differentiation-specific cues. By using this innovative approach, Naldini's team could overcome the immunological barrier to stable gene transfer, one of the major hurdles to successful gene therapy, establish long-term correction of hemophilia in mouse and dog models and induced active tolerance to the transgene.

Naldini's laboratory has also pioneered the use of engineered Zinc-finger nucleases to target vector integration and edit the human genome. These studies have opened the way to *correct*, rather than replace genes, a potentially revolutionary approach that may substantially expand the scope and power of genetic manipulation.

SELECTED PUBLICATIONS.

Original Research Articles

1. A. Annoni,Brown B.D.,Cantore A.,Sergi Sergi L.,**Naldini L.**,M.G.Roncarolo. In vivo Delivery of a MicroRNA Regulated Transgene Induces Antigen-specific Regulatory T Cells and Promotes Immunological Tolerance. **Blood** 2009 Dec 10;114(25):5152 61. Senior authors: LN and MGR.

   Times Cited:2

2. F. Pucci, MA Venneri, D Biziato, A Nonis, D Moi, A Sica, C Di Serio, **L Naldini**, M. De Palma. A distinguishing gene signature shared by tumor-infiltrating Tie2-expressing monocytes (TEMs), blood "resident" monocytes and embryonic macrophages suggests common functions and developmental relationships. **Blood** 2009 Apr 21. Jul 23; 114(4):901-14.Epub 2009 Apr 21.
   Corresponding/Senior authors: LN and MDP. Times Cited:15

3. M. Amendola,L. Passerini, F. Pucci, B. Gentner, R. Bacchetta, **L. Naldini.** Regulated and Multiple miRNA and siRNA Delivery into Primary Cells by a Lentiviral

Platform

   Molecular Therapy 2009 Mol Therapy 2009 Jun;17(6):1039-52.Epub 2009 Mar 17.

   Times Cited:1

4. E.Montini, D.Cesana, M.Schmidt, F.Sanvito,C. Bartholomae, M.Ranzani, F.Benedicenti, L.Sergi Sergi, A.Ambrosi, M. Ponzoni, C.Doglioni, C.Di Serio, C.von Kalle & **L.Naldini.** The genotoxic potential of retroviral vectors is strongly modulated by vector design and integration site selection in a mouse model of hematopoietic stem cell gene therapy. **Journal of Clinical Investigation** 2009, Apr;119(4):964-75. Epub 2009 Mar 23. Times

Cited:46

5. B. Gentner, G. Schira, A. Giustacchini, M. Amendola, B.D. Brown, M. Ponzoni and **L. Naldini.** Stable Knockdown of microRNA in Vivo by Engineered Lentiviral Vectors. **Nature Methods** 2009 Jan;6(1):63-6. Epub 2008 Nov 30. Times Cited:25

6. M. De Palma R. Mazzieri, L.S. Politi, F. Pucci , E. Zonari, S. Mazzoleni, G. Sitia, D. Moi, M.A. Venneri, S. Indraccolo, A. Falini, L.G. Guidotti, R. Galli, and **L. Naldini** .Tumor-targeted interferon-α delivery by Tie2-expressing monocytes inhibits tumor growth and metastasis. **Cancer Cell** 2008, Oct 7;14(4):299-311. Times Cited:27

7. F.R. Santoni de Sio, A. Gritti, P. Cascio , M.Neri , M.Sampaolesi , C.Galli, J.Luban, **L. Naldini.** Lentiviral Vector Gene Transfer is limited by the proteasome at post-entry steps in various Types of Stem Cells. **Stem Cells.** 2008 Aug;26(8):2142-52. Epub 2008 May 15. Times Cited:7

8. Lombardo, P. Genovese, C. M. Beausejour, S. Colleoni, Y.-L. Lee, K. A. Kim, D. Ando, F. Urnov, C. Galli, P. D. Gregory, M. C. Holmes, **L. Naldini**. Gene Editing in Human Stem Cells Using Zinc Finger Nucleases and Integrase-Defective Lentiviral Vector Delivery. **Nature Biotechnology.** 2007 Nov;25(11):1298-306. Epub 2007 Oct.28 . Times Cited:114

9. B. Brown, B. Gentner, A. Cantore,S. Colleoni, M.Amendola, A. Zingale, A.Baccarini, G. Lazzari, C. Galli, **L.Naldini**. Endogenous microRNA can be broadly exploited to regulate transgene expression according to tissue, lineage and differentiation state. **Nature Biotechnology.** 2007 Dec;25(12):1457-1467.Epub 2007 Nov.16 Times Cited:62

10. Brown BD, Cantore A, Annoni A, Sergi Sergi L, Lombardo A, Della Valle P,D'Angelo A, **Naldini L.** A microRNA-regulated lentiviral vector mediates stable correction of Hemophilia B mice. **Blood.** 2007 Dec 15;110(13):4144-52. Epub 2007 Aug.28.Times Cited:32

11. M.A.Venneri, M.De Palma, M.Ponzoni, F.Pucci, C.Scielzo, E.Zonari, R.Mazzieri, C.Doglioni, **L.Naldini.** Identification of Proangiogenic TIE2-Expressing Monocytes (TEMs) in Human Peripheral Blood and Cancer. **Blood**. 2007 Jun 15; 109(12):5276-85. Epub 2007 Feb 27.
Times Cited:56

12. Brown BD, Sitia G, Annoni A, Hauben E, Sergi Sergi L, Zingale A,Roncarolo MG, Guidotti LG,**Naldini L.** In vivo administration of lentiviral vectors triggers a type I interferon response that restricts hepatocyte gene transfer and promoters vector clearance. **Blood.** 2007 Apr 1;109(7):2797-805;Epub 2006 Dec 19. Times cited:31

13. A.Biffi, A. Capotondo, S. Fasano, U. del Carro, S. Marchesini, H. Azuma, M.C. Malaguti, S. Amadio, R.Brambilla, M. Grompe, C. Bordignon, A.Quattrini and **L. Naldini**. Gene Therapy of metachromatic leukodystrophy reverses neurological damage and deficits in mice. **Journal of Clinical Investigation.** 2006 Nov;116 (11):3070-82.Times Cited:39

14. E. Montini, D. Cesana, M. Schmidt, F. Sanvito, M. Ponzoni, L. Sergi Sergi, F. Benedicenti, C. Bartholomae, A. Ambrosi, C. Di Serio, C. Doglioni, C. von Kalle, and **L. Naldini**. Hematopoietic Stem Cell Gene Transfer in a Tumor-Prone Mouse Model Uncovers Low Genotoxicity of Lentiviral Vector Integration. **Nature Biotechnology**. 2006 Jun;24(6):687-96 Times Cited:183

15. F.R. Santoni de Sio, P. Cascio, A. Zingale, M. Gasparini and **L. Naldini**. Proteasome Activity Restricts Lentiviral Gene Transfer in Hematopoietic Stem Cells and is Down-Regulated by Cytokines that Enhance Transduction. **Blood.** 2006 Jun 1;107(11):4257-65. Times Cited:22

16. B.D. Brown, M.A. Venneri, A. Zingale, L. Sergi Sergi and **L. Naldini**. Endogenous microRNA Regulation Suppresses Transgene Expression in Hematopoietic Lineages and Enables Stable Gene Transfer. **Nature Medicine**. 2006 May;12(5):585-91. Times Cited:77

17. M. De Palma, M.A. Venneri, R. Galli, L. Sergi Sergi, L.S. Politi, M. Sampaolesi and **L. Naldini**.Tie2 Identifies a Hematopoietic Lineage of Pro-Angogenic Monocytes Required for Tumor Vessel Formation and a Mesenchymal Population of Pericyte Progenitors. **Cancer Cell.** 2005 Sep;8(3):211-26. Times Cited:243

18. M. De Palma, E. Montini, F. Santoni de Sio, A. Gentile, E. Medico, **L. Naldini**. Promoter Trapping Reveals Significant Differences in Integration Site Selection between MLV and HIV Vectors in Primary Hematopoietec Cells. **Blood.** 2005 Mar 15;105(6):2307-15. Times Cited:74

19. M. Amendola, M.A. Venneri, A. Biffi, E. Vigna, **L. Naldini**. Coordinate dual-gene transgenesis by Lentiviral Vectors Carrying Synthetic Bidirectional Promotors. **Nature Biotechnology**. 2005 Jan;23(1):108-16. Times Cited:63

20. E. Vigna, M. Amendola, F. Benedicenti, A.D. Simmons, A. Follenzi and **L. Naldini**. Efficient Tet-Dependent Expression of Human Factor IX in Vivo by a New Self-Regulating Lentiviral Vector. **Molecular Therapy.** 2005 May;11(5):763-75. Times Cited:24

21. A. Consiglio, A. Gritti, D. Dolcetta, A. Follenzi, C. Bordignon, F. H. Gage, A.L. Vescovi and **L. Naldini**. Robust in vivo gene transfer into adult mammalian neural stem cells by lentiviral vectors. **Proceedings National Academy of Sciences of USA**. 2004 Oct12;101(41):14835-40. Times Cited: 73

22. P. Michieli, M. Mazzone, C. Basilico, S. Cavassa, A. Sottile, **L. Naldini** and P.M. Comoglio. Targeting the Tumor and its Microenvironment by a Dual-Function Decoy Met Receptor. **Cancer Cell.** 2004 Jul;6(1):61-73. Times Cited:95

23. A. Follenzi, M Battaglia, A. Lombardo, A. Annoni, MG Roncarolo and **L. Naldini.** Targeting Lentiviral Vector Expression to Hepatocytes Limits Transgene-Specific Immune Response and Establishes Long-Term Expression of Human Antihemophilic Factor IX in Mice. **Blood.** 2004 May 15;103(10):3700-9. Times Cited:80

24. A. Biffi, M. De Palma, A. Quattrini, U. Del Carro, S. Amadio, I. Visigalli, M. Sessa, S. Fasano, R. Brambilla, S. Marchesini, C. Bordignon and **L. Naldini**. Correction of Metachromatic Leukodystrophy in the Mouse Model by Transplantation of Genetically Modified Hematopoietic Stem Cells. **The Journal of Clinical Investigation**. 2004 Apr;113(8):1118-29. Times Cited:88

25. M.M. De Palma, M.A. Venneri and **L. Naldini**. In vivo Targeting of Tumor Endothelial Cells by Systemic Delivery of Lentiviral Vectors. **Human Gene Therapy**. 2003 Aug 10;14(12):1193-206. Times Cited:48

26. S. Cavalieri, S. Cazzaniga, M. Geuna, Z. Magnani, C. Bordignon, **L. Naldini** and C. Bovini. Human T Lymphocytes Transduced by Lentiviral Vectors in the absence of TCR-Activation Maintain an Intact Immune Competence. **Blood**. 2003 Jul 15;102(2):497-505. Times Cited:53

27. M. De Palma, M.A. Venneri, C. Roca and **L. Naldini**. Targeting Exogenous Genes to Tumor Angiogenesis by Transplantation of Genetically modified Hematopoietic Stem Cells. **Nature Medicine**. 2003 Jun;9(6):789-95. Times Cited:218

28. L.L. Ailles, M. Schmidt, F. Santoni de Sio, H. Glimm, S. Cavalieri, S. Bruno, W. Piacibello, C. Von Kalle and **L. Naldini**. Molecular Evidence of Lentiviral Vector Mediated Gene Transfer into Human Self-Renewing, Multi-Potent, Long-Term NOD/SCID Repopulating Hematopoietic Cells. **Molecular Therapy**. 2002 Nov;6(5):615-26. Times Cited:63

29. E. Vigna, S. Cavalieri, L. Ailles, M. Geuna, R. Loew, H. Bujard and **L. Naldini**. Robust and Efficient Regulation of Transgene Expression in Vivo by Improved Tetracycline-Dependent Lentiviral Vectors. **Molecular Therapy**. 2002 Mar;5(3):252-61. Times Cited:71

30. A. Follenzi, G. Sabatino, A. Lombardo, C. Boccaccio and **L. Naldini.** Efficient Gene Delivery and Targeted Expression to Hepatocytes In Vivo by Improved Lentiviral Vectors. **Human Gene Therapy**. 2002;13: 243-260. Times Cited:135

31. D. Farson, R. Witt, R. McGuinness, T. Dull, M. Kelly, J. Song, R. Radeke, A. Bukovsky, A. Consiglio and **L. Naldini**. A New-Generation Stable Inducible Packaging Cell Line for Lentiviral Vectors. **Human Gene Therapy**. 2001 May 20;12(8):981-97. Times Cited:60

32. A.Consiglio, A. Quattrini, S. Martino, J.C. Bensadoun, D. Dolcetta, A. Trojani, G. Benaglia, S. Marchesini, V. Cestari, A. Oliverio, C. Bordignon and **L. Naldini**. In Vivo Gene Therapy of Metachromatic Leukodystrophy by Lentiviral Vectors: Correction of Neuropathology and Protection Against Learning Impairments in Affected Mice. **Nature Medicine**. 2001 Mar;7(3):310-6. Times Cited:127

33. A.Follenzi, L.E. Ailles, S. Bakovic, M. Geuna and **L. Naldini**. Gene transfer by lentiviral vectors is limited by nuclear translocation and rescued by HIV-1 pol sequences. **Nature Genetics**. 2000 Jun;25(2):217-22. Times Cited:472

34. G. Guenechea, O. Gan, T. Inamitsu, C. Dorrell, D. Pereira, M. Kelly, **L. Naldini** and J.E. Dick. Transduction of CD34+CD38- bone marrow and cord blood-derived SCID-Repopulating Cells with third-generation Lentiviral Vectors. **Molecular Therapy**. 2000 Jun;**1**(6):566-73. Times Cited:120

35. F. Park , K. Ohashi, W. Chiu, **L. Naldini** and M. A. Kay. Efficient lentiviral transduction of liver requires cell cycling in vivo. **Nature Genetics**. 2000 Jan;24(1):49-52. Times Cited:152

36. A. Bukovsky, J.-P. Song, and **L. Naldini**. Interaction of Human Immunodeficiency Virus-Derived Vectors with Wild-Type Virus in Transduced Cells. **Journal of Virology**. 1999 Aug;73(8):7087-92. Times Cited:72

37. S.S. Case, M.A. Price, C.T. Jordan, X.J. Yu, L.J. Wang, G. Bauer, D.L. Haas, D.Xu, R.Stripecke, **L. Naldini**, D.B. Kohn and G. M. Crooks. Stable Transduction of Quiescent CD34+CD38- Human Hematopoietic Cells by HIV-1 Based Lentiviral Vectors. **Proceedings National Academy of Sciences of the USA**. 1999 Mar 16;96(6):2988-93. Times Cited:260

38. R. Zufferey, T. Dull, R.J. Mandel, A. Bukovsky, D. Quiroz, **L. Naldini** and D.Trono. Self-Inactivating Lentiviral Vector For Safe And Efficient In Vivo Gene Delivery. **Journal of Virology**. 1998 Dec;72(12):9873-80. Times Cited:667

39. T.Dull, R. Zufferey, M. Kelly, R.J. Mandel, M. Nguyen, D. Trono and **L. Naldini**. A Third-Generation Lentiviral Vector with a Conditional Packaging System. **Journal of Virology.** 1998 Nov;72(11):8463-71. Times Cited:725

40. R. Zufferey, D. Nagy, R. J. Mandel, **L. Naldini** and D. Trono. Multiply Attenuated Lentiviral Vector Achieves Efficient Gene Delivery In Vivo. **Nature Biotechnology**. 1997 Sep;15(9):871-5.

Times Cited:738

41. **L.Naldini**, U. Blömer, F. H. Gage, D. Trono and I. M. Verma. Efficient Transfer, Integration, and Sustained Long-Term Expression of the Transgene in Adult Rat Brains Injected with a Lentiviral Vector. **Proceedings National Academy of Sciences of the USA**. 1996 Oct 15;93(21):11382-8. Times Cited:610

42. **L. Naldini**, U. Blömer, P. Gallay, D. Ory, R. Mulligan, F. H. Gage, I. M. Verma and D. Trono. In vivo Gene Delivery and Stable Transduction of Nondividing Cells by a Lentiviral Vector. **Science.** 1996 Apr 12;272(5259):263-7. Times Cited:2094

43. **L. Naldini**, E. Vigna, A. Bardelli, A. Follenzi, F. Galimi and P. M. Comoglio. Biological Activation of pro-HGF (Hepatocyte Growth Factor) by Urokinase is Controlled by a Stoichiometric Reaction. **The Journal of Biological Chemistry.** 1995 Jan 13;270(2):603-11. Times Cited:166

44. **L. Naldini**, L. Tamagnone, E. Vigna, M. Sachs, G. Hartmann, W. Birchmeier, Y. Daikuhara, H. Tsubouchi, F. Blasi and P.M. Comoglio. Extracellular Proteolytic Cleavage by Urokinase is required for Activation of Hepatocyte Growth Factor/Scatter Factor. **EMBO Journal**. 1992;11:4825-4833. Times Cited:414

45. **L. Naldini**, K.M. Weidner, E. Vigna, G. Gaudino, A. Bardelli, C. Ponzetto, R.P. Narsimhan, G. Hartmann, R. Zarnegar, G.K. Michalopoulos, W. Birchmeier and P.M. Comoglio. Scatter Factor and Hepatocyte Growth Factor are Indistinguishable Ligands for the Met Receptor. **EMBO Journal**. 1991;10:2867-2878. Times Cited:478

46. **L. Naldini**, E. Vigna, R. Ferracini, P. Longati, L. Gaudino, M. Prat, and P.M. Comoglio. The Tyrosine Kinase Encoded by the MET Proto-oncogene is Activated by Autophosphorylation. **Molecular and Cellular Biology.** 1991;11:1793-1803. Times Cited:138

47. **L. Naldini**, E. Vigna, R. Narsimhan, G. Gaudino, R. Zarnegar, G.K. Michalopoulos and P.M. Comoglio. Hepatocyte Growth Factor (HGF) Stimulates the Tyrosine Kinase Activity

of the Receptor Encoded by the Proto-Oncogene c-MET. **Oncogene**. 1991;6:501-504. Times Cited:572

48. **L. Naldini**, D. Cirillo, T.W. Moody, P.M. Comoglio, J. Schlessinger and R. Kris. Solubilization of the Receptor for the Neuropeptide Gastrin-Releasing Peptide (Bombesin) with Functional Ligand Binding Properties. **Biochemistry.** 1990 May 29;29(21)5153-60. Times Cited:14

49. **L. Naldini**, A. Stacchini, D. Cirillo, M. Aglietta, F. Gavosto and P.M. Comoglio. Phosphotyrosine Antibodies Identify the P210c-abl Tyrosine Kinase and Proteins Phosphorylated on Tyrosine in Human Chronic Myelogenous Leukemia Cells. **Molecular and Cellular Biology.** 1986 May;6(5):1803-11. Times Cited:10

Invited Reviews and Commentaries

1. **M. De Palma, L. Naldini.** Antagonizing metastasis. **Nature Biotechnology** 2010 Apr;28(4):331-2.

2. **L. Naldini.** A comeback for Gene Therapy. **Science** 2009 Nov 6;326(5954):805-6. Times Cited:5

3. B.D. Brown and **L. Naldini**. Exploiting and antagonizing miRNA regulation for therapeutic and experimental applications. **Nature Reviews Genetics** 2009 Aug.;10(8):578-85. Times Cited:26

4. M. De Palma, C. Murdoch, M.A. Venneri, **L. Naldini**, C.E. Lewis. Tie2-expressing Monocytes: Regulation of Tumor Angiogenesis and Therapeutic Implications. **Trends Immunology.** 2007 Dec;28(12):545-50.Epub 2007 Nov 5. Times Cited:31

5. C.E. Lewis, M. De Palma, **L. Naldini**. Tie2-expressing Monocytes: Regulation by Hpoxia and angioprotein. **Cancer Research.** 2007 Sep15;67(18):8429-32. Times Cited:24

6. M. De Palma and **L. Naldini**. Role of Haematopoietic Cells and Endothelial. Progenitors in Tumor Angiogenesis. **BBA-Reviews on Cancer**. 2006 Aug;1766(1):159-66. Times Cited:38

7. **L. Naldini**. Inserting Optimism into Gene Therapy. **Nature Medicine**. 2006 Apr;12(4):386-8.
Times Cited:5

8. A.Biffi and **L. Naldini**. Gene Therapy of Storage Disorders by Retroviral and Lentiviral Vectors. **Human Gene Therapy**. 2005 Oct;16(10):1133-42. Times Cited:15

9. M.A. Kay, J. Glorioso and **L. Naldini**. Viral Vectors for Gene Therapy: the art of turning infectious agents into vehicles of therapeutics. **Nature Medicine**. 2001 Jan;7(1):33-40.
Times Cited:459

Exhibit 2

R. Rieger · A. Michaelis · M.M. Green

Glossary of Genetics

Classical and Molecular

[redacted] Fifth Edition

Springer-Verlag

1st edition (German) 1954
2nd edition (German) 1958
Russian edition 1967
3rd edition (English) 1968
Polish edition 1974
4th edition (English) 1976
Spanish edition 1982
Chinese edition 1988
5th edition (English) 1991

With 13 Tables

ISBN 3-540-52054-6 Springer-Verlag Berlin Heidelberg New York
ISBN 0-387-52054-6 Springer-Verlag New York Berlin Heidelberg

Printed in Germany

Typesetting: Graphischer Großbetrieb Pößneck
Printing and bookbinding: Clausen & Bosse, Leck
31/3145-543210 – Printed on acid-free paper

prokaryotes which invaded primitive eukaryotic cells and became established as permanent symbionts in the cytoplasm.

end point mutation (Demerec 1946) — any experimentally induced → gene mutation in bacteria which due to delayed manifestation (→ cell division lag; mutational lag) becomes recognizable only after a series of cell divisions (→ zero point mutation).

end product inhibition — a biological control mechanism in sequential → enzyme systems in which the accumulation of the final product of a sequence of metabolic reactions causes the inhibition of its own formation. End product inhibition is an example of "negative feedback control mechanisms", which may be grouped into competitive and noncompetitive ones. (1) **Competitive mechanisms:** The inhibition of an early enzyme in a sequence by the end product of the enzyme sequence is competitive with the substrate of an early enzyme. There is structural similarity between end product and substrate. (2) **Noncompetitive mechanism:** The end product of the enzyme sequence inhibits an earlier enzyme in the sequence in some manner other than by competition with the substrate for the catalytic site. The end product, which is inhibitory, becomes bound to an independent site of the enzyme. This causes a conformational change in the enzyme, altering its catalytic activity. The second site is specific for binding the end product of the multienzyme sequence. Enzymes of this types are called → allosteric.

enforced heterozygosity (Muller 1917, 1918) — → heterozygous.

enforced outbreeding — deliberate avoidance of mating between relatives (→ inbreeding).

enhancement — increased yield of viruses or increased cytopathic effects, or both, after mixed infection of cells by unrelated animal viruses, at least one of which is noncytocidal.

enhancer — → modifier gene.

enhancer sequence (Khoury and Gruss 1983) — any of a class of cis-acting short DNA sequences that increase transcriptional activity (transcriptional enhancer) of eukaryotic genes. Transacting cellular factors are required for activity of at least some e. sequences. Many RNA polymerase II transcription enhancer sequences contain a conserved octamer sequence motif ATTTGCAT with which two transcription factors interact.

Activation of transcription of a gene linked in cis to the e.s. occurs in a relatively orientation- and location-independent fashion. E.sequences display tissue and species specificity and activate transcription over large distances (→ silencer sequence).

enneaploid — of a → polyploid with nine chromosome sets in the somatic cells.

entry exclusion (Harada et al. 1961) — the phenomenon (= surface e.) whereby a number of conjugal → plasmids create a cell surface barrier to conjugal entry of genetically distinguishable derivatives of themselves. E.e. is quite distinct from → plasmid incompatibility, which operates at post-DNA penetration stages.

entry site — the ribosome site available for the initial binding of → transfer RNA during → genetic translation. In the process of initiation (→ initiation complex), → initiator tRNA binds first at this so-called A site and is then translocated to a second aminoacyl tRNA molecule which could now bind to the e.s.

enucleate — of a → cell lacking a → nucleus. The removal of the nucleus with very little cytoplasm is called enucleation and represents a classical method for investigating interactions between nucleus and cytoplasm (→ nuclear transplantation).

environment — the combination of all conditions external to the → genome that influence its expression and structure, and thereby the organism. Normally, an organism is adapted to the various different factors of its e. But the heterogeneity of the e. can have profound effects on evolutionary processes and can influence directly the genetic structure of → populations (→ environmental mutagenesis).

environmental mutagenesis — a field of chemical → mutagenesis studying potential mutagenic effects of, e.g., food additives, pesticides, drugs, cosmetics, and industrial compounds (= genetic toxicology). Many of these agents require "activation" following ingestion before any mutagenesis is manifest. The genetic changes resulting from environmental mutagens